


# Exhibit 16



# *Ovarian Cancer: Etiology, Risk Factors, and Epidemiology*

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**Abstract:** Little is known regarding the early aspects of ovarian carcinogenesis. As a consequence, the identification of women at risk for the disease is based primarily on clinical grounds, with family history being the most important risk factor. In this review, we will discuss the various hypotheses regarding ovarian etiology and pathogenesis. In addition, we will discuss the epidemiology of ovarian cancer, including hereditary, reproductive, hormonal, inflammatory, dietary, surgical, and geographic factors that influence ovarian cancer risk.

**Key words:** ovarian cancer, epidemiology, risk factors, etiology, pathogenesis

## ***Introduction***

Epithelial ovarian cancer remains a highly lethal malignancy. It is the fourth to fifth leading cause of cancer deaths among women in the United States and causes more than 140,000 deaths annually in women worldwide. Despite intensive research efforts over the past decade directed toward improved detection and

treatment of ovarian cancer, the majority of women diagnosed with ovarian cancer succumb to the disease. Progress in the fight against ovarian cancer has been hampered by a number of factors. These include late diagnosis, the absence of highly curative chemotherapy, and a high degree of molecular heterogeneity in ovarian tumors, a finding that is a direct consequence of the large tumor burden typical in most patients at the time of presentation. Despite the challenges, substantial progress has been made in our understanding of ovarian cancer biology, the potential mechanisms underlying protective factors, and our ability to identify women at increased risk of the disease. This is translating into more effective methods of prevention and treatment, and a corresponding fall in ovarian cancer incidence and mortality rates.<sup>1</sup>

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*The authors declare that they have nothing to disclose*

## ***Etiology***

Because of the intra-abdominal location of the ovary as well as the preponderance

of advanced disease at presentation typical of most ovarian cancers, it has been difficult to characterize changes in the ovarian surface epithelium (OSE) consistent with intraepithelial neoplasia.<sup>2</sup> Thus, little is known regarding the very early molecular and genetic events associated with ovarian carcinogenesis. As a consequence, the etiology of ovarian cancer remains poorly understood, and even the cell of origin of epithelial ovarian cancer has not been conclusively defined. A common but unproven hypothesis is that ovarian cancers arise in OSE cell-lined inclusion cysts, which are nests of OSE that are entrapped in the ovarian stroma, and subjected to the stimulative influence of stromal growth factors. Evidence to support the OSE as the source of ovarian cancer includes: (1) the finding of activation of cancer preventive molecular pathways specifically in the OSE by the oral contraceptive pill (OCP), a known ovarian cancer preventive<sup>3,4</sup>; (2) description of premalignant, dysplastic changes in the OSE using classic pathologic criteria<sup>5</sup>; (3) colocalization of dysplastic histologic changes with either loss of tumor suppressor activity or overexpression of cyclooxygenase 2 in the OSE of high-risk ovaries<sup>6,7</sup>; and (4) the finding of a transition in some early ovarian cancers from a nonmalignant to malignant OSE.<sup>8</sup>

Recently, an alternative hypothesis has been proposed, which suggests that the cell of origin for ovarian cancer may involve cells that have originated in the fallopian tube.<sup>9–13</sup> This hypothesis is speculative, but supported by the finding that most ovarian cancers have a histology similar to that of the fallopian tube. In addition, fallopian tube cancer risk is markedly elevated in women with BRCA-related hereditary risk of ovarian cancer, and an unusually high incidence of histologic and molecular signatures associated with dysplasia have been identified in the fimbriated end of the fallopian tube in prophylactic oophorectomy specimens from women at high

risk.<sup>13,14</sup> Further, careful examination of the fallopian tube in women with serous pelvic carcinoma has demonstrated a high incidence of endosalpinx involvement, or of coexistent tubal carcinomas, with similar alterations in p53 noted in the pelvic and fallopian tube lesions, suggesting that the lesions might be genetically related.<sup>15,16</sup> An unusually high incidence of p53 signatures has been noted even in the fimbriated ends of fallopian tubes removed for noncancerous indications in women at presumed population-based risk of ovarian cancer.<sup>17</sup> It is possible that the fimbriated end of the fallopian tube may be susceptible to neoplasia when exposed to dysplastic cells shed from the OSE or even in response to ovarian stromal factors released during ovulation.

## **PATHOGENESIS**

It has been commonly believed that ovulation, with its associated disruption and subsequent repair of the ovarian epithelium, can lead to the acquisition of genetic damage in ovarian epithelial cells and, in turn, to ovarian cancer in susceptible individuals.<sup>18–20</sup> The “incessant ovulation” hypothesis for ovarian cancer is supported by a large volume of epidemiologic evidence linking ovulation with ovarian cancer risk<sup>18,21–29</sup> and by the finding that spontaneous ovarian cancers arise frequently in poultry hens, which ovulate daily.<sup>30</sup> Of note, alterations in p53 are common in epithelial ovarian cancer. In addition, in human as well as chicken ovarian adenocarcinomas, the incidence of p53 alterations correlates with the number of lifetime ovulatory events.<sup>31</sup> It is possible that ovulatory events predispose the ovarian epithelium to alterations in p53, leading to defective repair of DNA and thus ovarian cancer susceptibility. The mechanism(s) by which these changes could potentially lead to neoplastic transformation of the fallopian tube is unclear.

Under the incessant ovulation model, reproductive and hormonal factors such as OCP use and pregnancy have been presumed to alter ovarian cancer risk mainly through their inhibitory impact on ovulation. Although this hypothesis is attractive, it fails to explain completely the marked reduction in the degree of ovarian cancer risk associated with factors such as pregnancy and OCP use. For example, both of these factors confer a degree of ovarian cancer protection that is much greater than what would be expected simply based on the number of ovulatory cycles that are inhibited.<sup>21,23</sup> In addition, pregnancy is associated with a reduced risk of ovarian cancer even in women who are known to have ovulatory dysfunction and for whom the pregnant state has little impact on the number of lifetime ovulatory cycles.<sup>32</sup> Further, some studies have reported a relationship between increasing risk of epithelial ovarian cancer and increasing time since last birth.<sup>33,34</sup> These data support the hypothesis that reproductive and or hormonal factors impact ovarian cancer risk through additional biological mechanisms unrelated to ovulation inhibition.<sup>35</sup> Indeed, in addition to incessant ovulation, there is evidence in support of alternative hypotheses that have been proposed to explain ovarian cancer pathogenesis, including (1) the gonadotropin hypothesis, which purports that circulating gonadotropins stimulate the ovarian epithelium and promote neoplastic transformation,<sup>36</sup> (2) the hormonal hypothesis which suggests that reproductive hormones can interact directly with the ovarian epithelium to promote (estrogens and androgens) or protect against (progestins) carcinogenesis,<sup>3,4,37</sup> and (3) the inflammation hypothesis which argues that inflammatory mediators released either during ovulation or concomitant with disease processes such as endometriosis can damage the epithelium in the ovary and or fallopian tube.<sup>38,39</sup> Although none of these

hypotheses can fully explain all ovarian cancers, it is likely that they all play a role, and that ovarian cancer pathogenesis is a multifactorial process, involving a complex interplay of biological events related to ovulation, inflammation, and gonadal/hormonal factors.

## ***Risk Factors and Epidemiology***

As a consequence of the fact that most ovarian cancers present in an advanced stage, the molecular or tissue biomarker changes associated with the very early aspects of ovarian epithelial carcinogenesis are not well known. Moreover, even if tissue biomarker changes predictive of neoplastic transformation of the OSE were known, the relative inaccessibility of the ovary would make it difficult to use this knowledge clinically to identify women at increased risk of the disease. In addition, despite extensive serum biomarker research, there is still a lack of robust serum biomarkers that can be used reliably to identify, in a timely way, the majority of women who are destined to develop ovarian cancer.<sup>40</sup> Thus, in contrast to other cancers such as that of the colon or cervix, there is insufficient tissue or other biomarker information to allow clinicians to identify women at risk, and risk identification is based primarily on epidemiologic factors (Table 1).

## **HEREDITARY**

One of the most consistent and significant risk factors for ovarian cancer is a family history of ovarian cancer, particularly in first-degree relatives.<sup>41,42</sup> Schildkraut et al<sup>43</sup> examined the family histories of ovarian cases and controls who had been identified in conjunction with the Cancer and Steroid Hormone (CASH) Study in the early 1980s. The risks of ovarian cancer in first-degree and second-degree relatives of women with ovarian cancer were found to be increased 3.6- and 2.9-fold, respectively,

**TABLE 1. Risk Factors for Epithelial Ovarian Cancer**

| Increased   | Decreased  | Indeterminate                                    |
|---|--|--|
| Hereditary<br>Family history of ovarian cancer<br>Personal history of breast cancer<br>Alteration in <i>BRCA1</i> or <i>BRCA2</i><br>Lynch syndrome | Reproductive<br>Multiparity<br>Breastfeeding<br><br>Hormonal<br>Oral contraceptives<br>Progestins<br>Surgery<br>Hysterectomy<br>Tubal ligation | Fertility drugs<br>Exercise<br>Cigarette smoking |
| Reproductive<br>Advanced age<br>Nulligravity<br>Infertility   |  |  |
| Hormonal<br>Early age at menarche<br>Late age at natural menopause<br>Hormone replacement therapy<br>Estrogen<br>Androgens                          |  |  |
| Inflammatory<br>Perineal talc exposure<br>Endometriosis<br>Pelvic inflammatory disease  |  |  |
| Lifestyle<br>Obesity  |  |  |
| Geography<br>Extremes in latitude   |  |  |

compared with women with no family history of ovarian cancer. Analysis of the CASH data also revealed that a family history of either breast or ovarian cancer increased the risk of both cancers in first-degree relatives.<sup>43–45</sup> The discovery of the *BRCA1* and *BRCA2* cancer susceptibility genes confirmed the hypothesis that a fraction of ovarian cancers arise in women with a genetic predisposition. It is now thought that about 10% to 12% of women with ovarian cancer carry germline mutations in the *BRCA1* or *BRCA2* genes.<sup>46–50</sup> An additional 2% to 3% are from families with hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome. These families carry mutations in DNA repair genes and have as high as 10% to 13% lifetime risk of ovarian cancer, although colorectal, gastric, and endometrial cancers are more commonly seen.<sup>51,52</sup> Even among families with identical *BRCA1* or *BRCA2* mutations, there is

heterogeneity with respect to the fraction of breast versus ovarian cancer that manifest and the age at onset. This suggests that genetic susceptibility is modified by other genetic or environmental factors. Cardinal features of hereditary cancer risk include a familial pattern suggestive of autosomal dominant inheritance, early onset, an excess of bilaterality (breast), multiple primaries (breast-ovary), and in the case of Lynch syndrome, an excess of cancers of the gastrointestinal and genitourinary tracts. Women with a familial pattern consistent with a significant risk of ovarian cancer should be referred for counseling and consideration of genetic testing (Table 2).<sup>53</sup>

### ***BRCA***

Families with *BRCA1* and *BRCA2* mutations represent the formerly separate syndromes of site-specific familial ovarian cancer and heredity breast/ovarian

**TABLE 2. Factors Suggestive of an Inherited Predisposition to Breast and/or Ovarian Cancer for Whom Referral for Genetic Evaluation Should Be Considered**

|  |
|--|
| <b><i>BRCA</i>*</b>  |
| Personal history of both breast and ovarian cancer   |
| Personal history of ovarian cancer and a close relative with breast cancer at $\leq 50$ y or ovarian cancer at any age   |
| History of ovarian cancer at any age combined with Ashkenazi Jewish ancestry   |
| History of breast cancer at $\leq 50$ y and a close relative with ovarian or male breast cancer at any age   |
| Women of Ashkenazi Jewish ancestry and breast cancer at $\leq 40$ y  |
| Women with a first-degree or second-degree relative with a known <i>BRCA1</i> or <i>BRCA2</i> mutation   |
| Women with bilateral breast cancer (particularly if the first cancer was at $\leq 50$ y)   |
| Women with breast cancer at $\leq 50$ y and a close relative with breast cancer at $\leq 50$ y   |
| Women of Ashkenazi Jewish ancestry with breast cancer at $\leq 50$ y   |
| Women with breast or ovarian cancer at any age and 2 or more close relatives with breast cancer at any age (particularly if at least 1 breast cancer was at $\leq 50$ y) |
| <b>Lynch</b>   |
| Women with endometrial or colorectal cancer who have   |
| At least 3 relatives with a Lynch/HNPCC-associated cancer (colorectal cancer, cancer of the endometrium, small bowel, ureter, or renal pelvis) in 1 lineage              |
| One affected individual should be a first-degree relative of the other 2   |
| At least 2 successive generations should be affected   |
| At least 1 HNPCC-associated cancer should be diagnosed before age 50   |
| Women with synchronous or metachronous endometrial and colorectal cancer with the first cancer diagnosed before age 50   |

\*Peritoneal and fallopian tube cancer should be considered as part of the spectrum of the hereditary breast/ovarian cancer syndrome.

HNPCC indicates hereditary nonpolyposis colorectal cancer.

Adapted from Schorge et al.<sup>53</sup> [Close relative is defined as a first, second, or third degree relative (ie, mother, sister, daughter, aunt, niece, grandmother, granddaughter, first cousin, great grandmother, great aunt)].

cancer.<sup>54</sup> Two thirds of these cancers are associated with alterations in *BRCA1* and the other third with alterations in *BRCA2*. The *BRCA* genes are tumor suppressor genes that play a role in the maintenance of genome integrity; they are involved in repair of double-strand DNA breaks, control of cell cycle checkpoint responses, and chromosomal segregation.<sup>55</sup> Affected individuals inherit an altered allele as well as normal wild-type allele for the *BRCA* genes. Loss of the wild-type alleles through either loss of heterozygosity or other somatic mutations in individuals with germline mutations in *BRCA1* and *BRCA2* leads to increases in genomic instability and tumorigenesis.<sup>55</sup>

The lifetime ovarian and breast cancer risks for women with *BRCA* mutations greatly surpasses that in the general population. Individuals from high-risk families with *BRCA1* mutations have an 87% cumulative risk of breast cancer by the age of 70. The lifetime risk of ovarian cancer in *BRCA1* mutation carriers is approximately 30% overall, but has been estimated to be as high as 44% in high-penetrance families.<sup>56</sup> The risk for breast and ovarian cancer is lower in women with mutations in *BRCA2*, with a 27% lifetime risk of ovarian cancer and an 84% risk of breast cancer.<sup>57</sup> Only a proportion of the women who carry *BRCA1* and *BRCA2* mutations develop ovarian cancer; the incomplete penetrance is thought to be due to multiple factors including the specific type and or location of the mutation, the status of modifying genes, epigenetic phenomena, and gene-environment interactions.<sup>58,59</sup> Of note, the estimated frequency of *BRCA* mutations in the general population is relatively low (1 in 300 to 1 in 800 individuals in the United States), but is considerably higher in those of Ashkenazi Jewish heritage (1 in 50).<sup>60</sup> Thus, in women with breast or ovarian cancer, those of Ashkenazi Jewish heritage are significantly more likely to harbor an alteration in *BRCA1* or *BRCA2*.

### ***Lynch Syndrome (HNPCC)***

A strong family history of early onset colon or endometrial cancer, or multiple malignancies of the gastrointestinal and genitourinary system should alert clinicians to the possibility of Lynch syndrome.<sup>53</sup> In addition to a significant lifetime risk of developing colon cancer, HNPCC patients have an increased risk of ovarian (12%) and endometrial cancers (40% to 60%).<sup>61</sup> These patients carry a mutation in the DNA mismatch repair genes MSH2, MLH1, PMS1, and PMS2, leading to genomic instability and cancer risk.<sup>62</sup> Similar to *BRCA*-related cancers, it has been observed that women with Lynch syndrome develop ovarian cancer at a younger age than women with sporadic ovarian cancer, with a mean age of 48. In half of the cases, ovarian and/or endometrial cancers occur as many as 5 or more years before the onset of colon cancer, thereby being the sentinel event alerting clinicians to the possible risk of HNPCC.<sup>63</sup> Patients who have developed malignancies suspicious for Lynch syndrome often undergo genetic assessment in a stepwise fashion starting with screening of tumor (uterus or colon) for mismatch repair defects.<sup>53</sup> Patients with abnormalities on immunohistochemical evaluation of MLH1, MSH2, MSH6, and PMS2 protein expression or microsatellite instability will then typically undergo full sequence analysis of relevant genes as directed by immunohistochemical results.

## **REPRODUCTIVE**

### ***Parity***

Case-control evidence has consistently shown that pregnancy lowers ovarian cancer risk. One pregnancy lowers ovarian cancer risk by as much as one third and the reduction in risk increases with each additional pregnancy.<sup>21,23–27</sup> The protective effect lingers for as long as

1 to 2 decades, but then wanes with increasing time since last birth.<sup>33,34</sup> In addition, pregnancy at a later age is more protective than pregnancy early in life. In fact, a pregnancy after the age of 35 is twice as protective against ovarian cancer as a pregnancy before the age of 25. It has been proposed that this would suggest a protective effect of pregnancy that is unrelated to effects on ovulation, and supporting the notion that pregnancy may clear premalignant or damaged cells from the ovary.<sup>64–65</sup> Infertility is associated with a 2-fold increased relative risk (RR) of ovarian cancer. Data on the impact of fertility drug use on risk have been inconsistent, perhaps because of the confounding influences of infertility and pregnancy on ovarian cancer risk.<sup>66–69</sup> Of note, similar to women who are fertile, women treated for infertility who successfully achieve a live birth benefit from a reduction in ovarian cancer risk.

### ***OCP Use***

Numerous case-control studies have shown that OCP use is associated with a decreased risk of ovarian cancer.<sup>21,70</sup> Three or more years of OCP use reduces the risk of developing epithelial ovarian cancer by 30% to 50%.<sup>22,71</sup> The association increases with the duration of use and appears to be independent of inherent ovarian cancer risk.<sup>23,72</sup> Furthermore, the duration of protection effect lasts for more than 10 to 20 years after the last use. These data are quite similar to the epidemiologic data related to parity, suggesting that parity and OCP use may share a common biological mechanism underlying their ovarian cancer protective effect.

### ***Breastfeeding***

Although the results of published studies are inconsistent, the weight of the published evidence suggests that breastfeeding lowers ovarian cancer risk. Danforth evaluated the impact of breastfeeding on ovarian cancer risk in a large study of 391

ovarian cancer cases and over 149,000 total participants.<sup>73</sup> Analysis was confined to parous women to evaluate the impact of breastfeeding independent of parity. The median duration of breastfeeding among women who breastfed was 9 months. As compared with never breastfeeding, any breastfeeding was not associated with a statistically significant reduction in ovarian cancer risk. However, among those women who breastfed for 18 months or more, a significant 34% decrease in ovarian cancer risk was noted as compared with never breastfeeding. A similar protective effect of breastfeeding was noted in a case-control study of parous women in New Hampshire, but only for women who had either breastfed all children, or the last born child.<sup>74</sup> No protective effect was found when the last born child was not breastfed. The authors speculated that breastfeeding may “reset pregnancy-related influences on ovarian cancer risk.” In contrast, Jordan found a modest 2% reduction in ovarian cancer risk associated with breastfeeding, and no additional benefit from individual lactation episodes >12 months. In addition, the protective effect did not hold for serous borderline or mucinous subtypes, but was generally maintained for other histologic subtypes of ovarian cancer.<sup>75</sup>

## **HORMONAL**

There is mounting evidence that the ovarian epithelium is a hormonally responsive target organ whose biology can be impacted strongly by the local hormonal environment. The normal ovarian epithelium expresses receptors for most members of the steroid hormone superfamily, including estrogens, progestins, retinoids, vitamin D, and androgens. In addition, the ovarian epithelium contains gonadotropin receptors and nonhormonal targets such as the cyclooxygenase pathway. There is therefore the potential for reproductive and environmental factors

to have an impact on ovarian cancer risk through a direct biological interaction of hormonal and nonhormonal agents on the ovarian epithelium. Recent studies have indeed shown that reproductive hormones can have potent biological effects directly on the ovarian epithelium, thus impacting ovarian cancer risk. Progestins, for example, have been shown to induce apoptosis, one of the most important molecular pathways in vivo for the prevention of cancer and a pathway that mediates the action of many known chemopreventive agents. It has been proposed that progestin-mediated apoptotic effects may be a major mechanism underlying the ovarian cancer protective effects of OCP use and pregnancy (a high progestin state). Similarly, retinoids, vitamin D, and nonsteroidal anti-inflammatory drugs may have biological effects on the ovarian epithelium that are cancer preventive, whereas estrogens and androgens may have stimulatory effects on the ovarian epithelium, leading to an increased ovarian cancer risk.<sup>3,4,37,76</sup>

## **Gonadotropins**

As early as the 1980s, Cramer proposed the gonadotropin hypothesis as a potential mechanism underlying ovarian carcinogenesis.<sup>24</sup> He proposed that elevated circulating levels of gonadotropins related to either the menopause or ovulatory events might stimulate the OSE and promote neoplastic transformation. The biological mechanisms underlying the gonadotropin hypothesis have not been well characterized, however, and the theory has fallen short in fully explaining the impact of hormonal and reproductive events on ovarian cancer risk. Recently, an excellent review by Choi has summarized the evidence in support of or against the gonadotropin hypothesis, and the published data have generally yielded inconsistent findings.<sup>77</sup> For example, although gonadotropin receptors have been shown to be expressed in the normal

ovarian epithelium and ovarian neoplasms, an association between serum levels of gonadotropins and ovarian cancer has not been conclusively established. Similarly, the known reduction in ovarian cancer risk associated with pregnancy and OCP use, conditions where gonadotropins are suppressed, supports the gonadotropin hypothesis; yet hormone replacement therapy, which also suppresses gonadotropins, is associated with an increase in ovarian cancer risk. Finally, gonadotropins have been shown to both inhibit and stimulate carcinogenesis in vitro, and animal data have been similarly inconsistent.

### ***Progestins***

The biological mechanism underlying the protective effect of OCP use has historically been presumed to be related to the inhibitory effect of OCPs on ovulation, and, in turn, to a lessening in the extent of ovulation-induced genetic damage accumulated in the OSE. Recent animal data, however, suggest that the OCP may have a profound, direct chemopreventive effect in the OSE, mediated by the progestin component. A 3-year study in primates has demonstrated that the progestin component of an OCP has a potent apoptotic effect on the ovarian epithelium, providing support for the hypothesis that OCPs may lower ovarian cancer risk through progestin induction of cancer preventive molecular pathways in the ovarian epithelium.<sup>3,4</sup> The apoptosis pathway is arguably one of the most important in vivo mechanisms for cancer prevention. Activation of apoptosis leads to the efficient disposal of cells that have undergone irreparable genetic damage and that are prone to neoplastic transformation.<sup>78</sup> It is thus a key molecular pathway for the elimination of premalignant cells in vivo. It is a biological mechanism associated with many known chemopreventive agents,<sup>79–86</sup> and pharmacologic agents that selectively enhance apoptosis have been shown to lower the risk of a variety of cancers in animals and in

humans.<sup>87</sup> In addition, in both animal models of cancer as well as in humans, the efficacy of cancer preventive agents has been shown to correlate with the degree of apoptosis induced.<sup>87–90</sup> Conversely, mutations in the genes involved in the apoptosis pathway have been shown to be associated with enhanced cancer risk.<sup>91</sup> The finding that progestins activate this critical pathway in the ovarian epithelium raises the possibility that progestin-mediated apoptotic effects, and not solely ovulation inhibition as has been previously assumed, may underlie the reduction in ovarian cancer risk associated with routine OCP use and pregnancy.

A growing body of published human data is supportive of the notion that a biological effect related to progestins may be a major mechanism underlying the cancer preventive effect for both the OCP as well as pregnancy, which confers potent protection against subsequent ovarian cancer and which is associated with high serum progesterone levels:

- (a) An analysis of the data from the CASH, has demonstrated that use of progestin-potent OCPs confers greater protection against ovarian cancer than use of OCPs containing weak progestin formulations.<sup>92</sup>
- (b) Further support for progestins as ovarian cancer preventives has come from an analysis of data from the WHO by Risch, demonstrating a 60% reduction in the risk of nonmucinous ovarian cancer in women who have ever used Depo-medroxyprogesterone acetate, a progestin-only contraceptive.<sup>37</sup> Progestin-only contraceptives do not reliably inhibit ovulation. Thus, the 60% reduction in ovarian cancer risk from a progestin-only contraceptive is further evidence that progestins have a direct chemopreventive effect on the ovary.
- (c) In addition, epidemiologic evidence has suggested that twin pregnancy may be more protective against

subsequent ovarian cancer than singleton pregnancy. Previously, it was presumed that women who have twins would be at greater risk of ovarian cancer, presumably due to an increased likelihood of more lifetime ovulatory events as compared with women who do not have twins, and the notion that increased ovulation would confer greater risk of ovarian epithelial damage. Because women with twin pregnancy have higher progesterone levels than women with singleton pregnancy, it has been proposed that the data regarding twin pregnancy are supportive of the notion of a biological effect of progesterone as conferring ovarian cancer protection, and that the effect is dose dependent.<sup>64</sup>

- (d) Finally, pregnancy at a later age is more protective than pregnancy early in life, and pregnancy after the age of 35 is twice as protective against ovarian cancer as a pregnancy before the age of 25. It has been proposed that this would suggest a protective effect of pregnancy that is unrelated to effects on ovulation, and supporting the notion that pregnancy may clear premalignant or damaged cells from the ovary.<sup>64,65</sup> Reproductive factors such as pregnancy and OCP use may thus impact ovarian cancer risk not only through inhibition of ovulation, but also through a progestin-mediated chemopreventive effect that clears genetically damaged cells from the ovarian epithelium.

### **Estrogens**

Data regarding the impact of estrogens on ovarian cancer risk are mainly derived from case-control series examining the impact of OCP use or hormone replacement therapy on ovarian cancer risk. As discussed above, use of estrogen/progestin combination OCPs has been shown to consistently lessen ovarian cancer risk.<sup>71</sup>

Of note, however, in primates receiving OCPs, estrogens have been shown to partly abrogate the effect of progestins on chemopreventive endpoints such as apoptosis in the OSE, suggesting that estrogens may counteract the cancer preventive effect of progestins.<sup>3,4</sup> Published evidence in postmenopausal women would support this conclusion. Several large case-control studies suggest that estrogen replacement therapy increases ovarian cancer risk 2-fold, and that the addition of progestins to hormone replacement therapy partly neutralizes this enhanced risk.<sup>93-97</sup> Whether or not estrogen replacement therapy increases the risk for all ovarian cancers, or selectively promotes the development of specific histologic subtypes of ovarian cancer is unclear. For example, an increase in risk for endometrioid ovarian tumors has been reported among women who have used postmenopausal estrogen replacement.<sup>97,98</sup> A more recent study, however, has shown that menopausal hormone replacement use conferred an increased risk for all histologic subtypes of ovarian cancer except for mucinous, where risk was reduced.<sup>99</sup>

### **Androgens**

It has been proposed that androgens may be associated with increased ovarian cancer risk, but the evidence is not conclusive.<sup>37,100</sup> Data in support of a link between androgens and ovarian cancer risk include: (1) androgen receptors (ARs) are expressed in the OSE, thereby providing a means by which androgens can have a direct biological effect in the organ; (2) most ovarian cancers express AR, and antiandrogens inhibit ovarian cancer growth; (3) oral contraceptives, potent ovarian cancer preventives, significantly lower ovarian androgen production; (4) ovarian cancer risk is increased in conditions such as polycystic ovary syndrome, which is associated with elevated serum androgen levels; (5) use of androgenic agents such as testosterone or danazol may increase ovarian cancer risk.<sup>101,102</sup> In contrast, however, increased

activity of the AR gene may inhibit ovarian carcinogenesis. In addition, a recent case-control study evaluating clinical surrogates for an androgenic milieu such as a history of polycystic ovary syndrome, acne or hirsutism failed to demonstrate that androgen excess is associated with increased ovarian cancer risk.<sup>101</sup> Finally, use of androgenic OCPs does not increase ovarian cancer risk as compared with nonandrogenic OCPs.<sup>103</sup>

## INFLAMMATION

Ness was the first to propose that inflammatory factors might be involved in ovarian carcinogenesis.<sup>104</sup> In her comprehensive review in 1999, she noted that the incessant ovulation and gonadotropin hypotheses failed to adequately explain the enhanced risk of ovarian cancer associated with talc use, endometriosis and pelvic inflammatory disease (PID), as well as the protective effects associated with hysterectomy and tubal ligation. A growing body of evidence suggests that the ovarian epithelium and fallopian tube are exposed chronically to an inflammatory milieu related to the normal functions of ovulation and menstruation.<sup>105</sup> Pro-inflammatory cytokines are present in ovulatory fluid and also in menstrual effluent that comes into contact with the fallopian tube. These same cytokines are markedly elevated in epithelial ovarian cancers. In addition, inflammatory mediators are markedly increased in disease states such as endometriosis and PID. Recently, elevated serum levels of C-reactive protein have been shown to be associated with an increased subsequent risk of ovarian cancer.<sup>106,107</sup> In addition, in a prospective case-control study of 230 women with ovarian cancer and 432 individually matched controls nested within three prospective cohorts, prediagnostic circulating levels of inflammatory cytokines, such as the interleukins, have been shown to be elevated in women who subsequently developed ovarian cancer. These data provide more direct

evidence that inflammation may be associated with ovarian cancer risk.<sup>108</sup> Interestingly, OCPs, which as described above, markedly lower ovarian cancer risk, confer a number of biological effects that can mitigate inflammatory influences in the genital tract, including inhibiting ovulation, lowering the risk of PID, and reversing endometriosis.<sup>109</sup>

## Talc

Evidence demonstrating an association between talc use and an increased risk of ovarian cancer suggests that environmental toxins can enter the lower genital tract and migrate upward through the uterus and fallopian tubes to enter the peritoneal cavity and act as ovarian carcinogens. Talcum powder was first implicated in the risk of ovarian cancer in the 1960s when it was found to be biologically similar to asbestos which is a known carcinogen. Subsequent studies in animals and humans demonstrated not only that talc deposited in the gynecologic tract could reach the ovaries, but also the finding of talc particles in ovarian neoplasms.<sup>110</sup> Subsequent case-control studies of talc use and risk of ovarian cancer have shown a strong association, including a meta-analysis of 16 studies that included 11,933 women demonstrating a 33% increased risk of ovarian cancer.<sup>111–115</sup>

## Endometriosis

Endometriosis has been consistently shown to be associated with an increased risk of ovarian cancer, with odds ratios of approximately two.<sup>104,116</sup> The underlying mechanism is not fully characterized. It has been proposed that chronic inflammation can lead to neoplastic transformation of endometriotic implants. In addition, it is possible that the endometriotic state leads to a relative progesterone “resistance”, thereby mitigating the potential protective effects of the hormone.<sup>117,118</sup> The most common histologic subtypes of ovarian cancer associated

with endometriosis are clear cell and endometrioid carcinomas.<sup>119</sup>

### **PID**

PID occurs as predominantly a consequence of sexually transmitted diseases and manifests clinically as a marked inflammatory process involving the uterus, fallopian tubes, and ovaries. Limited case-control evidence suggests an increased risk of ovarian cancer among women who have had PID.<sup>120,121</sup> The association appears to be most pronounced in women who have had PID at a young age, or who are infertile, which is also an ovarian risk factor. In the largest study to date, with over 67,000 women with PID and over 135,000 controls, the adjusted hazard ratio for ovarian cancer in women with PID was 1.92, increasing to 2.46 in women who had had 5 or more episodes of PID. The adjusted hazard ratio was higher for women aged 35 or younger.<sup>121</sup>

### **SURGERY**

Hysterectomy and tubal ligation are associated with a reduction in the risk of developing ovarian cancer. In a meta-analysis of 12 case-control studies, hysterectomy (without oophorectomy or salpingectomy) was associated with a 34% reduction in the risk of ovarian cancer.<sup>29</sup> Women who underwent a tubal ligation also had a 34% risk reduction compared with women who did not.<sup>122</sup> The protective effect of surgery also extends to women at hereditary risk of ovarian cancer. A case-control study by the Hereditary Ovarian Cancer Clinical Study Group has shown that tubal ligation lowered the rate of ovarian cancer in women with *BRCA1* alterations by 60%.<sup>123</sup> The combination of tubal ligation and OCP use reduced the risk even further. Of note, no protective effect of tubal ligation was seen among carriers of the *BRCA2* mutation. The mechanism for the protective effect of tubal ligation and

hysterectomy is not known, but theoretically could be explained by blockage of access of environmental carcinogens to the ovaries. Another proposed mechanism is that surgery to remove uterus or fallopian tubes may affect the ovarian circulation or plasma hormone levels in ways that lower ovarian cancer risk.<sup>124</sup> Finally, if the fallopian tube is indeed the source of some ovarian cancers, then removing some of the tube may be expected to lower cancer risk.

### **LIFESTYLE**

#### ***Obesity***

It is likely that obesity increases the risk of ovarian cancer, but the degree of effect is modest. A systematic review reported a small association between body mass index (BMI) >30 and ovarian cancer risk with an odds ratio of 1.3 [95% confidence interval (CI), 1.1-1.5].<sup>125</sup> In the Cancer Prevention Study, a prospective cohort study of 495,477 women followed for 16 years, a relationship was noted between high BMI and ovarian cancer mortality.<sup>126</sup> The RR of death from ovarian cancer among women with a BMI of 35 to 40 was 1.51 compared with those of normal weight. Findings from the Nurses' Health Study indicated a 2-fold increased risk of premenopausal ovarian cancer associated with a high BMI.<sup>127</sup> In addition, a meta-analysis showed an association between obesity and ovarian cancer with a 40% increase in risk in the heaviest versus the lightest women in population-based case-control studies.<sup>128</sup> A recent study by Leitzman prospectively followed 94,525 patients over a 7-year period.<sup>129</sup> Overall, the women with a BMI > 30 were 1.26 times more likely to have developed ovarian cancer, though those findings were not statistically significant. Among a subgroup of women who had never used hormone replacement therapy, the women who were obese were 1.83 times more likely to develop ovarian cancer. In

women who had used hormone replacement therapy, there was no association between obesity and ovarian cancer. The authors speculated that obesity is associated with enhanced ovarian cancer risk through a hormonal mechanism. Obesity is known to increase adrenal secretion of androgens, and is generally associated with an increased endogenous production of estrogens.<sup>130</sup>

### **Diet**

Numerous studies have attempted to identify dietary factors that may influence ovarian cancer risk. Overall, the results have been inconsistent or conflicting. The balance of the evidence has failed to conclusively demonstrate that consumption of any macronutrient or micronutrient significantly alters ovarian cancer risk. A case-control study in Italy comparing 455 cases with ovarian cancer to 1385 age-matched controls revealed an increased RR for ovarian cancer associated with meat consumption of >7 portions versus less than 4 portions per week (RR 1.6; 95% CI, 1.2-2.12) and butter versus fat consumption (RR 1.9; 95% CI, 1.20-3.11). Dietary risk factors that decreased risk included whole-grain bread and pasta consumption.<sup>131</sup> A larger prospective cohort study of 29,083 women in the United States found that egg consumption of 2 to 4 times per week as well as increased intake of carbohydrates and dairy increased the RR of developing ovarian cancer, whereas consumption of green leafy vegetables significantly decreased risk (RR 0.44, 95% CI, 0.25-0.79), but there was no association with dietary fat, as well as intake of meats, breads cereals, and starches and ovarian cancer risk.<sup>132</sup>

Studies evaluating the intake of specific foods or food groups on the subsequent development of ovarian cancer have similarly yielded inconsistent results. In one study, protective foods included olive and vegetable oils, fish, peas, beans, and

lentils.<sup>133</sup> Vegetable consumption was found to be protective in one study<sup>134</sup> but another study that examined the effect of consumption of vegetables and fruits noted no benefit.<sup>135</sup> In another large study, risk of ovarian cancer was studied after consumption of fruit and vegetables. There was no association found between high consumption of fruits and vegetables and ovarian cancer risk.<sup>136</sup> A study in 2006 suggested that milk and milk products are associated with an increased ovarian cancer risk.<sup>137</sup> However, the Netherlands Cohort Study on Diet and Cancer which followed 62,573 women for 11.3 years and included 252 cases with ovarian cancer found no association between lactose and dairy intakes and the development of ovarian cancer.<sup>138</sup>

In attempt to further clarify dietary associations with ovarian cancer risk, 2 studies evaluated general dietary patterns as opposed to specific foods. Overall diet was evaluated in the prospective California Teachers Study.<sup>139</sup> A total of 97,292 women completed a baseline dietary assessment of which 311 developed epithelial ovarian cancer. Five major dietary patterns were compared: (1) plant-based; (2) high protein/high fat; (3) high carbohydrate; (4) ethnic; (5) salad and wine. Although women who followed a plant-based diet had a slightly higher risk of ovarian cancer (RR 1.65, 95% CI, 1.07-2.54), the authors concluded that their results did not show convincing associations between dietary patterns and ovarian cancer risk. A recent study published in 2011 evaluated the association between a Healthy Eating Index and ovarian cancer.<sup>140</sup> The Healthy Eating Index reflects adherence to current USDA dietary Guideline for Americans. This population-based case-control study had a total of 205 women with ovarian cancer and 390 controls. Based on their results, the authors concluded that neither individual food groups nor dietary quality showed potential for preventing ovarian cancer.

### **Exercise**

There is no firm relationship between exercise and ovarian cancer risk. Studies to date are small and generally inconclusive, with results ranging from suggesting no association, to a finding of a modest benefit from exercise, to even a possible adverse effect of vigorous exercise on ovarian cancer risk.<sup>141–144</sup> Pan et al<sup>145</sup> examined survey responses from over 400 women with ovarian cancer and over 2100 healthy women from The Canadian National Enhanced Cancer Surveillance System. Women who reported moderate levels of recreational physical activity or who held jobs with moderate or strenuous physical activity had a reduced risk of ovarian cancer with an odds ratio of 0.67 (0.50 to 0.88). A large study from the Netherlands Cohort Study consisting of 62,573 women who were surveyed regarding their physical activity yielded similar conclusions. Two hundred fifty-two cases of ovarian cancer were identified after 11.3 years of follow-up. Compared with women who exercised < 30 minutes per day, women who spent > 60 minute per day in moderate exercise had a RR of 0.78 for the development of ovarian cancer. Women who spent > 2 hours per week on recreational biking and walking had an even lower risk (RR 0.65; 95% CI, 0.41–1.01) compared with women who did no exercise.<sup>146</sup> In contrast, in the very large Nurses Health Study, although moderate activity was found to be protective against subsequent ovarian cancer, frequent vigorous exercise was associated with increased risk.<sup>143</sup> The underlying mechanism(s) potentially mediating the effects of exercise on ovarian risk are not well known. Hormonal changes associated with physical activity can cause anovulation and decrease the risk of obesity thereby lowering estrogens and risk, but possibly increase gonadotropins which may increase risk.

### **Cigarette Smoking**

The effect of smoking on ovarian cancer risk has not been well defined. The most

intriguing finding has been an association between current or past smoking and an increase in mucinous ovarian cancer, although the association does not apply to other histologic subtypes.<sup>147–151</sup> The biological basis underlying any association between smoking and ovarian cancer is not well understood. Nicotine and its metabolites have been identified in ovarian tissue.<sup>152</sup> Thus, it is plausible that these agents can cause direct DNA damage in the OSE. In addition, cigarette smokers have been found to have higher circulating levels of gonadotropins and androgens, both of which can have adverse effects on risk. On the other hand, smokers may have earlier onset of menopause which would be expected to lower risk.<sup>153–155</sup>

### **GEOGRAPHY**

Worldwide, there is a geographic distribution for ovarian cancer, with increasing incidence commensurate with latitudinal distance from the equator.<sup>156</sup> The same pattern holds in the United States where there is a significant north-south gradient, favoring a higher ovarian cancer risk in northern versus southern latitudes in the United States. Lefkowitz has correlated population-based data regarding ovarian cancer mortality in large cities across the United States with geographically based long-term sunlight data reported by the National Oceanic and Atmospheric Administration, demonstrating a statistically significant inverse correlation between regional sunlight exposure and ovarian cancer mortality risk.<sup>157</sup> Given that sunlight induces production of previtamin D in the skin, it is interesting to speculate that vitamin D might confer protection against ovarian cancer by direct biological effects in the nonmalignant ovarian epithelium, similar to that induced by progestins. For example through induction of apoptosis and/or transforming growth factor- $\beta$  in the ovarian epithelium,

vitamin D may cause the selective removal of nonmalignant, but genetically damaged ovarian epithelial cells.<sup>158,159</sup> A small case-control study supports the notion that vitamin D confers ovarian cancer prevention, at dosages of vitamin D easy to achieve through the diet. As compared with a low dietary intake of vitamin D, a high dietary intake of vitamin D was associated with a 50% reduction in ovarian cancer risk.<sup>160</sup>

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